REMARKS

Claims 1-20 are pending; all of the claims stand rejected following the Office Action mailed on April 20, 2000. An Amendment after final rejection, filed on July 12, 2000, was not entered as stated in the Advisory Action mailed August 21, 2000. Applicants have filed a Request for Continuing Examination (RCE) herewith and by this amendment present amendments to the claims and comments with regard to the cited art to place the application in better condition for continuing examination.

Claims 1, 6, 9, and 19 have been amended. No new matter has been added by these amendments and support for the amendments can be found throughout the specification, particularly at page 9, line 25 to page 10, line 3, page 24, lines 2-5, page 25, lines 16-22, and page 26, lines 7 to 10. Entry of this amendment before examination continues on the merits of this application is respectfully requested.

Rejections under 35 U.S.C. § 102(b)

Claims 19 and 20 stand rejected under 35 U.S.C. § 102(b) based on the disclosure of Saunders et al. (U.S. Pat. No. 5,066792).

To anticipate an invention, the cited reference must disclose an invention that does not differ from the claimed invention, as viewed by a person of ordinary skill in the field of the invention. *Scripps Clinic & Research Fndn. v. Genetech*, Inc., 927 F.2d 1565, 18 USPQ2d 1001 and 1896 (Fed. Cir. 1991).

The invention of amended claim 19 includes the steps of (a) providing a biological sample comprising unpurified RNA, (b) mixing the biological sample with a solution "consisting essentially of" a buffer, about 150 mM to about 1 M of a soluble salt, and about 0.5% to about 1.5% (v/v) of a non-ionic detergent, and with a solid support to which is joined an immobilized oligonucleotide which forms a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions, (c) separating the hybridization complex joined to the solid support from unhybridized sample components, and (d) then washing the hybridization complex with a solution having sufficient salt concentration to maintain the

hybridization complex.

The claimed invention differs significantly from the RNA extraction method taught by Sauders et al. The claimed method uses "a non-ionic detergent" in the solution used in the mixing step. Saunders et al. do not use a non-ionic detergent and, instead, use a well-known anionic detergent (col. 5, lines 34-36, Sarcosyl is the trade name of N-lauroylsarcosine, which is well known to those skilled in the art). The cell lysis procedure of Saunders et al. releases chromosomal DNA, as shown by the description of multiple centrifugation and resuspension steps to remove DNA (col. 5, lines 52-56). Although amended claim 19 no longer contains the phrase "sufficient to release RNA ... without causing viscosity due to release of chromosomal DNA", Applicants' specification teaches this aspect of the invention. The Examiner (Paper 10) stated that "any detergent will cause some viscosity since absent evidence to the contrary, any lysing of a cell will release both types of nucleic acids, DNA and RNA" but provides no support for this statement. Applicants respectfully request that the Examiner either provide a reference to support this conclusion or request that the Examiner provide a declaration under 37 C.F.R. § 1.107(b) in support of this statement, to avoid possible "procedural default" and having the assertion established as "admitted prior art." *See In re Sun*, 31 U.S.P.Q.2d 1451, 1455 (Fed. Cir. 1993). Based on the different types of detergents in the claimed invention and the cited reference, the Saunders et al. reference does not anticipate this element of the claim.

Applicants' claimed method mixes the biological sample with a solution containing about 150 mM to about 1 M soluble salt. In contrast, Sauders et al. disclose using a solution containing 75 mM NaCl, and thus does not anticipate this element of the claim.

Because Saunders et al. do not teach every element of the claimed invention, the reference cannot anticipate the present invention. Therefore, Applicants respectfully request that the rejections of claims 19 and 20 under 35 U.S.C. 102(b) be withdrawn.

Rejections under 35 U.S.C. § 103

Claims 1-18 stand rejected under 35 U.S.C. §103(a), based on the combination of Eskola et al. (Clin. Biochem., 1994, 27:373-379), Kacian et al. (US Pat. 5,399,491) and Saunders et al. in view of Rowley et al. (US Pat. 5,487,970), Morris et al. (US Pat. 5,529,925), von Lindern et al. (Molec. Cell. Biol., 1992, 12:3346-3355), Goddard et al. (Science, 29 Nov 1991, pp. 1371-1374), Gruenwald et al. (US Pat. 5,858,682) and Ohki et al. (US Pat. 5,580,727).

In this amendment, method claims 1, 6 and 9 have been amended. Claim 6 has been amended in the "contacting" step, to state that the solution "consisting essentially of" the specified components is used. Claims 1 and 9 have both been amended in the "hybridizing" step to clarify that hybridization occurs at "either the first probe binding site or the second probe binding site...."

A *prima facie* case of obviousness requires one to determine the content and scope of the prior art, ascertain the differences between the prior art and the claims at issue and determine the level of ordinary skill in the art. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). One of ordinary skill in the art of biotechnology generally has a relatively high level of training. An obviousness determination also requires consideration of whether the prior art would have suggested to one skilled in the art to make the claimed invention, and would have revealed a reasonable expectation of success in making the claimed invention. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Applicants' claim 1 includes the steps of a) providing a sample containing a first single-stranded fusion nucleic acid comprising a splice junction; b) contacting under nucleic acid amplification conditions the first single-stranded fusion nucleic acid, a first primer which hybridizes to the fusion nucleic acid at a first primer binding site located 3' to the splice junction site, and at least one nucleic acid polymerase activity; c) amplifying the fusion nucleic acid in an isothermal nucleic acid amplification reaction using the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the first single-stranded fusion nucleic acid that contains the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to sequence complementary to the first primer binding site; d) hybridizing the second nucleic acid strands with an oligonucleotide probe under hybridization conditions in which the probe hybridizes to either the first probe binding site or the second probe binding site, thereby forming a probe:target hybrid; and e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample. Claim 9 contains similar steps for detecting a fusion mRNA transcript resulting from a chromosomal translocation.

Applicants respectfully submit that the combined cited art does not suggest the claimed inventions for the following reasons, based on the teachings in the cited art.

1. Eskola et al.

Eskola et al. teach a method that requires two probes to bind to the target for detection: one probe that binds 5' of the splice junction and another probe that binds 3' of the splice junction (Abstract, lines 13-16; page 374, col. 1, lines 14-16 and p. 375, col. 2, lines 26-31). In the method taught by Eskola et al., detection requires one probe that binds the amplified sequence to a solid surface (e.g., by using a biotinylated probe that binds to streptavidin coated surface) and one labeled probe that detects the bound complex (page 375, col. 2, lines 23-48). For detection to function as taught by Eskola et al., both probes must bind to the amplified sequence. In contrast, Applicants' "hybridizing" steps, use a probe that binds to either the first probe binding site or the second probe binding site. Thus, Eskola et al. do not suggest Applicants' methods.

Although the Examiner has stated (in Paper 10) that it would have been within the ordinary skill of an artisan at the time the invention was made to detect the nucleic acid of the present claims using one of the probes taught by Eskola et al., this cannot be correct based on the teachings of Eskola et al. For example, if one used the probe of Eskola et al. that binds the amplified sequence to a solid surface (e.g.,

by using a biotinylated probe that binds to streptavidin coated surface), the result would be amplified sequence bound to a surface but lacking any means of detecting the bound amplified sequence. If one, instead, used the labeled probe taught by Eskola et al., in the absence of the probe that binds to the solid surface, one would have a solution containing labeled probe:amplified sequence complexes that would be indistinguishable from a solution merely containing the labeled probe. That is, if only one of the probes taught by Eskola et al. is bound to the sequence to be detected, the sequence cannot be detected. In fact, the disclosure of Eskola et al. teaches away from hybridizing to only one probe binding site because the method of Eskola et al. would be inoperative for detection if only one probe was hybridized. Thus, the Eskola et al. reference could not have suggested to one skilled in the art at the time the invention was made to use a single probe as claimed by the Applicants' methods. Therefore, the Eskola et al. reference cannot make the Applicants' claims obvious even if combined with the other cited art.

The Examiner (in Paper 10, page 3, last sentence) stated that "the two probes taught by Escola et al. for amplification of the nucleic acid target read on the limitations of the [present] claims for 'a first probe binding site ... and a second probe binding site' and therefor read on the claims as written." Applicants respectfully clarify that the claims refer to a "first probe binding site" and a "second probe binding site" (claims 1 and 9, steps c), which define the *positions* of sites relative to each other and other elements of the second nucleic acid strand. The present claims further make clear (claims 1 and 9, steps d) that the hybridizing step binds a probe to *only one* of these sites, i.e., at "either the first probe binding site or the second probe binding site." Therefore, Applicants respectfully argue that Eskola et al. do not suggest to one skilled in the art at the time the invention was made to make the present invention because Eskola et al. teach that both binding sites must be bound by the two probes taught by Eskola et al. Moreover, using the teachings of Eskola et al., one skilled in the art at the time the invention was made, would not have predicted success for Applicants' claimed invention because success would have required two probes to bind to the amplified sequence for detection based on the teachings of Eskola et al. Thus, if only one probe were used, one skilled in the art would not have predicted success for the claimed methods.

2. Kacian et al.

The Background section of Kacian et al. (column 1, lines 33-40) states that detection and quantitation of nucleic acid sequences is an increasingly important technique for a variety of applications including detecting genetic abnormalities or identifying genetic changes associated with cancer. The Examiner (in Paper 10) stated that this provides motivation that "suggests detection of chromosomal abnormalities in a sample" as presently claimed. Applicants, however, argue that the general statement in Kacian et al. of the importance of certain techniques for use in a variety of applications would not motivate one skilled in the art at the time the invention was made to make claimed invention, which uses a number of well defined steps reliant on specified structural elements. Nor would the disclosure of Kacian et al., which discloses isothermal amplification of nucleic acid, even if combined with other cited art, have revealed a reasonable expectation of success in making the claimed present invention to one skilled in the art at the time Applicants' invention was made.

In particular, the combination of Eskola et al., discussed above, even if combined with the Kacian et al. reference, would not have suggested the present claimed invention, because the invention suggested by that combination would have included amplification as taught by Kacian et al., and detection using the two-probe method as taught by Eskola et al.

Saunders et al.

Applicants respectfully refer the Examiner to the detailed discussion of the Saunders et al. reference in the comments above in response to the rejections under 35 U.S.C. §102(b). Because of the significant differences between the teachings of Saunders et al. and the claimed invention, Applicants respectfully argue that the combination of Eskola et al., Kacian et al. and Saunders et al. would not have suggested to one skilled in the art at the time the present invention was made to make the claimed invention.

Other Cited References

The Examiner has further cited Rowley et al., Morris et al., Gruenwald et al., von Lindern et al.,

Ohki et al. and Goddard et al. as teaching motivation in the art to detect specific genetic translocations claimed in dependent claims. These cited references disclose methods of detecting genetic rearrangements, specific fusion or chimeric sequences, and detection of a chimeric protein.

Rowley et al. teach methods of detecting genetic rearrangements that rely on detection of a gene fragment or message of aberrant size or pattern (Abstract, lines 10-17; col. 3, lines 55-60; col. 4, lines 1-7 and 32-42; col. 5, lines 4-8; col. 21, lines 43-46). The hybridization methods taught in this reference (e.g., Southern and Northern blotting) detect aberrant nucleic acids by using a "breakpoint-spanning" probe (col. 3, lines 17-19 and 31-33). This method is unlike Applicants' methods that, instead, hybridizes a probe to either the first probe binding site or the second probe binding site, both of which are not overlapping with the splice junction site (see step c in claims 1 and 9). The splice junction is another term for a "breakpoint" as is well known in the art. Thus, the "breakpoint-spanning" probe of Rowley et al. teaches away from Applicants' claimed invention.

Morris et al. teach detection of a NPM/ALK fusion sequence by nucleic acid amplification followed by detection using two probes: one that binds to the NPM sequence and one that binds to the ALK sequence. Binding of *both* probes detects the NPM/ALK fusion in the sample (col. 2, lines 1-30, particularly lines 28-30). As discussed above with regard to the Eskola et al. reference, this two-probe method teaches away from Applicants' claimed methods. Alternatively, Morris et al., teach using a *single probe that spans* the fusion sequence (col. 2, lines 31-33; col. 15, lines 65-67). As discussed above with regard to the Rowley et al. reference, this junction-spanning probe method teaches away from Applicants' claimed methods.

The Examiner cited Gruenwald et al., von Lindern et al., Ohki et al. and Goddard et al. as teaching various translocations. Applicants have acknowledged that various translocations are known in the art (e.g., page 2, line 15 to page 3, line 9), but such references do not suggest the claimed methods.

Gruenwald et al. teach detection of a chimeric protein by using monoclonal antibodies specific for the junction region or epitope of the chimeric protein (col. 2, lines 25-28, 33-34; col. 4, lines 30-36, 48-54).

von Lindern et al. describe isolation and characterization of a gene on chromosome 9, designated *set*, that was fused to a *can* gene, also on chromosome 9, in leukemic cells and produced a chimeric *set-can* transcript (page 3346, col. 2, last sentence before "MATERIALS AND METHODS" and p. 3351, col. 2, first paragraph). Neither of the references suggests the claimed invention even if combined with the Eskola, Kacian and Saunders references.

Ohki et al. teach amplification of a cDNA containing a t(8;21) fusion site and detection by using a probe that contains the fusion site (col. 3, line 55 to col. 4, line 7) or by amplifying a sequence containing the fusion site and detecting the amplified DNA with ethidium bromide staining on a gel (col. 4, lines 19-26). As discussed above with regard to the Rowley and Morris references, detection using probes that overlap the splice junction or fusion site teach away from Applicants' claimed invention.

Goddard et al. describe cDNA cloning and analysis of clones containing t(15;17) translocations (p. 1371, col. 1, first paragraph and col. 3, first full paragraph). That is, Goddard et al. reveal specific fusion sequences that one might want to detect. Even if combined with the teachings of the Eskola, Kacian and Saunders references, this reference does not make Applicants' claimed invention obvious for the same reasons as discussed above.

Based on the above comments, even if the Eskola et al. teachings were combined with the teachings of Kacian et al., and Saunders et al., in view of the teachings of the other cited art, the present invention would not have been suggested to one skilled in the art at the time the invention was made. Nor would these combined teachings have revealed a reasonable expectation of success in making the Applicants' claimed invention. Therefore, Applicants respectfully request that the rejections of claims 1-18 under 35 U.S.C. §103 be withdrawn because the Examiner has failed to present a *prima facie* case of obviousness.

CONCLUSION

In view of the foregoing amendments and remarks, the Applicants respectfully submit that the claims, as amended, are patentable and in condition for allowance. Accordingly, withdrawal of the rejections and allowance of the application is earnestly solicited. The undersigned has made a good-faith effort to address all the points raised in the last Office Action and to place the claims in condition for allowance. If the Examiner thinks that a telephone interview would further clarify issues discussed herein, the Examiner is invited to contact the undersigned at the number below.

Applicants believes there is no fee due in connection with the filing of this Amendment. However, if Applicants are in error and a fee is required, please debit Deposit Account No. 07-0835.

Respectfully submitted,

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